WO 97/38115
PCT/EP97/01741

PATENT COOPERATION TREATY

PCT

From the INTERNATIONAL BUREAU

To:

Patent- u. Lizenzabteilung K 801

HOECHST SCHERING AGREVO GMBH
Patente, Frankfurt Gebäude K 801
D-65926 Frankfurt am Main
ALLEMAGNE

27.OKT1997

Wv.
ablegen
Vert. wie Vorg./angegeb.

NOTICE INFORMING THE APPLICANT OF THE COMMUNICATION OF THE INTERNATIONAL APPLICATION TO THE DESIGNATED OFFICES

(PCT Rule 47.1(c), first sentence)

IMPORTANT NOTICE

Date of mailing (day/month/year)		
16 October 1997 (16.10.97)		
Applicant's or agent's file reference		
1996/M206		
International application No.	**International filing date** (day/month/year)	**Priority date** (day/month/year)
PCT/EP97/01741	08 April 1997 (08.04.97)	11 April 1996 (11.04.96)
Applicant		
HOECHST SCHERING AGREVO GMBH et al		

1. Notice is hereby given that the International Bureau has communicated, as provided in Article 20, the international application to the following designated Offices on the date indicated above as the date of mailing of this Notice:

   AU,BR,CA,CN,EP,IL,JP,KP,KR,NO,PL,SK,US

   In accordance with Rule 47.1(c), third sentence, those Offices will accept the present Notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Office(s).

2. The following designated Offices have waived the requirement for such a communication at this time:

   AL,AM,AP,AZ,BA,BB,BG,BY,CU,CZ,EE,GE,HU,IS,KG,KZ,LC,LK,LR,LT,LV,MD,MG,MK,MN,MX,NZ,
   OA,RO,RU,SG,SI,TJ,TM,TR,TT,UA,UZ,VN,YU

   The communication will be made to those Offices only upon their request. Furthermore, those Offices do not require the applicant to furnish a copy of the international application (Rule 49.1(a-bis)).

3. Enclosed with this Notice is a copy of the international application as published by the International Bureau on

   16 October 1997 (16.10.97) under No. WO 97/38115

REMINDER REGARDING CHAPTER II (Article 31(2)(a) and Rule 54.2)

If the applicant wishes to postpone entry into the national phase until 30 months (or later in some Offices) from the priority date, **a demand for international preliminary examination** must be filed with the competent International Preliminary Examining Authority before the expiration of 19 months from the priority date.

It is the applicant's sole responsibility to monitor the 19-month time limit.

Note that only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination.

REMINDER REGARDING ENTRY INTO THE NATIONAL PHASE (Article 22 or 39(1))

If the applicant wishes to proceed with the international application in the **national phase**, he must, within 20 months or 30 months, or later in some Offices, perform the acts referred to therein before each designated or elected Office.

For further important information on the time limits and acts to be performed for entering the national phase, see the Annex to Form PCT/IB/301 (Notification of Receipt of Record Copy) and Volume II of the PCT Applicant's Guide.

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. (41-22) 740.14.35

Authorized officer

J. Zahra

Telephone No. (41-22) 338.83.38

PCT

ANTRAG

Der Unterzeichnete beantragt, daß die vorliegende internationale Anmeldung nach dem Vertrag über die internationale Zusammenarbeit auf dem Gebiet des Patentwesens behandelt wird.

Vom Anmeldeamt auszufüllen

Internationales Aktenzeichen: PCT/EP 97 / 01741

Internationales Anmeldedatum: 08 APR 1997 (08. 04. 97)

Name des Anmeldeamts und "PCT International Application": EUROPEAN PATENT OFFICE PCT INTERNATIONAL APPLICATION

Aktenzeichen des Anmelders oder Anwalts *(falls gewünscht) (max. 12 Zeichen)*: 1996/M206

Feld Nr. I BEZEICHNUNG DER ERFINDUNG

Process for the production of plants with enhanced growth characteristics

Feld Nr. II ANMELDER

Name und Anschrift: *(Familienname, Vorname; bei juristischen Personen vollständige amtliche Bezeichnung. Bei der Anschrift sind die Postleitzahl und der Name des Staats anzugeben.)*

Hoechst Schering AgrEvo GmbH
Miraustraße 54
D-13509 Berlin
Deutschland

☐ Diese Person ist gleichzeitig Erfinder

Telefonnr.: 069-305-6537

Telefaxnr.: 069-35-7175

Fernschreibnr.: 4 1234 700 ho d

Staatsangehörigkeit (Staat): DE

Sitz oder Wohnsitz (Staat): DE

Diese Person ist Anmelder für folgende Staaten: ☐ alle Bestimmungsstaaten ☒ alle Bestimmungsstaaten mit Ausnahme der Vereinigten Staaten von Amerika ☐ nur die Vereinigten Staaten von Amerika ☐ die im Zusatzfeld angegebenen Staaten

Feld Nr. III WEITERE ANMELDER UND/ODER (WEITERE) ERFINDER

Name und Anschrift: *(Familienname, Vorname; bei juristischen Personen vollständige amtliche Bezeichnung. Bei der Anschrift sind die Postleitzahl und der Name des Staats anzugeben)*

DONN, Günter
Sachsenring 35
65619 Hofheim
Deutschland

Diese Person ist:

☐ nur Anmelder

☒ Anmelder und Erfinder

☐ nur Erfinder *(Wird dieses Kästchen angekreuzt, so sind die nachstehenden Angaben nicht nötig.)*

Staatsangehörigkeit (Staat): DE

Sitz oder Wohnsitz (Staat): DE

Diese Person ist Anmelder für folgende Staaten: ☐ alle Bestimmungsstaaten ☐ alle Bestimmungsstaaten mit Ausnahme der Vereinigten Staaten von Amerika ☒ nur die Vereinigten Staaten von Amerika ☐ die im Zusatzfeld angegebenen Staaten

☒ Weitere Anmelder und/oder (weitere) Erfinder sind auf einem Fortsetzungsblatt angegeben.

Feld Nr. IV ANWALT ODER GEMEINSAMER VERTRETER; ZUSTELLANSCHRIFT

Die folgende Person wird hiermit bestellt/ist bestellt worden, um für den (die) Anmelder vor den zuständigen internationalen Behörden in folgender Eigenschaft zu handeln als: ☐ Anwalt ☐ gemeinsamer Vertreter

Name und Anschrift: *(Familienname, Vorname; bei juristischen Personen vollständige amtliche Bezeichnung. Bei der Anschrift sind die Postleitzahl und der Name des Staats anzugeben.)*

Hoechst Schering AgrEvo GmbH
Patente, Frankfurt; Gebäude K 801
D-65926 Frankfurt am Main
Deutschland

Telefonnr.: 069-305-6537

Telefaxnr.: 069-35-7175

Fernschreibnr.: 41234700 ho d

☒ Dieses Kästchen ist anzukreuzen, wenn kein Anwalt oder gemeinsamer Vertreter bestellt ist und statt dessen im obigen Feld eine spezielle Zustellanschrift angegeben ist.

Blatt Nr. .....

Fortsetzung von Feld Nr. III WEITERE ANMELDER UND/ODER (WEITERE) ERFINDER

Wird keines der folgenden Felder benutzt, so ist dieses Blatt dem Antrag nicht beizufügen.

Name und Anschrift: *(Familienname, Vorname; bei juristischen Personen vollständige amtliche Bezeichnung. Bei der Anschrift sind die Postleitzahl und der Name des Staats anzugeben)*

ECKES, Peter
Am Flachsland 18
65779 Kelkheim
Deutschland

Diese Person ist:
- [ ] nur Anmelder
- [X] Anmelder und Erfinder
- [ ] nur Erfinder *(Wird dieses Kästchen angekreuzt, so sind die nachstehenden Angaben nicht nötig.)*

Staatsangehörigkeit (Staat): DE

Sitz oder Wohnsitz (Staat): DE

Diese Person ist Anmelder für folgende Staaten: [ ] alle Bestimmungsstaaten [ ] alle Bestimmungsstaaten mit Ausnahme der Vereinigten Staaten von Amerika [X] nur die Vereinigten Staaten von Amerika [ ] die im Zusatzfeld angegebenen Staaten

Name und Anschrift: *(Familienname, Vorname; bei juristischen Personen vollständige amtliche Bezeichnung. Bei der Anschrift sind die Postleitzahl und der Name des Staats anzugeben)*

MÜLLNER, Hubert
Stauffenstraße 1
65779 Kelkheim
Deutschland

Diese Person ist:
- [ ] nur Anmelder
- [X] Anmelder und Erfinder
- [ ] nur Erfinder *(Wird dieses Kästchen angekreuzt, so sind die nachstehenden Angaben nicht nötig.)*

Staatsangehörigkeit (Staat): DE

Sitz oder Wohnsitz (Staat): DE

Diese Person ist Anmelder für folgende Staaten: [ ] alle Bestimmungsstaaten [ ] alle Bestimmungsstaaten mit Ausnahme der Vereinigten Staaten von Amerika [X] nur die Vereinigten Staaten von Amerika [ ] die im Zusatzfeld angegebenen Staaten

Name und Anschrift: *(Familienname, Vorname; bei juristischen Personen vollständige amtliche Bezeichnung. Bei der Anschrift sind die Postleitzahl und der Name des Staats anzugeben)*

DUDITS, Genes
Bérkert u. 36/3
H-6726 Szeged
Ungarn

Diese Person ist:
- [ ] nur Anmelder
- [X] Anmelder und Erfinder
- [ ] nur Erfinder *(Wird dieses Kästchen angekreuzt, so sind die nachstehenden Angaben nicht nötig.)*

Staatsangehörigkeit (Staat): HU

Sitz oder Wohnsitz (Staat): HU

Diese Person ist Anmelder für folgende Staaten: [ ] alle Bestimmungsstaaten [ ] alle Bestimmungsstaaten mit Ausnahme der Vereinigten Staaten von Amerika [X] nur die Vereinigten Staaten von Amerika [ ] die im Zusatzfeld angegebenen Staaten

Name und Anschrift: *(Familienname, Vorname; bei juristischen Personen vollständige amtliche Bezeichnung. Bei der Anschrift sind die Postleitzahl und der Name des Staats anzugeben)*

PAULOVICS, Katalin
Fö út 2
H-3873 Gradna
Ungarn

Diese Person ist:
- [ ] nur Anmelder
- [X] Anmelder und Erfinder
- [ ] nur Erfinder *(Wird dieses Kästchen angekreuzt, so sind die nachstehenden Angaben nicht nötig.)*

Staatsangehörigkeit (Staat): HU

Sitz oder Wohnsitz (Staat): HU

Diese Person ist Anmelder für folgende Staaten: [ ] alle Bestimmungsstaaten [ ] alle Bestimmungsstaaten mit Ausnahme der Vereinigten Staaten von Amerika [X] nur die Vereinigten Staaten von Amerika [ ] die im Zusatzfeld angegebenen Staaten

[X] Weitere Anmelder und/oder (weitere) Erfinder sind auf einem zusätzlichen Fortsetzungsblatt angegeben.

Blatt Nr. ...3....

Fortsetzung von Feld Nr. III WEITERE ANMELDER UND/ODER (WEITERE) ERFINDER

Wird keines der folgenden Felder benutzt, so ist dieses Blatt dem Antrag nicht beizufügen.

Name und Anschrift: *(Familienname, Vorname: bei juristischen Personen vollständige amtliche Bezeichnung. Bei der Anschrift sind die Postleitzahl und der Name des Staats anzugeben)*

FEHER, Attila
József A. sgt. 134
H-6723 Szeged
Ungarn

Diese Person ist:
- [ ] nur Anmelder
- [X] Anmelder und Erfinder
- [ ] nur Erfinder *(Wird dieses Kästchen angekreuzt, so sind die nachstehenden Angaben nicht nötig.)*

Staatsangehörigkeit (Staat): HU

Sitz oder Wohnsitz (Staat): HU

Diese Person ist Anmelder für folgende Staaten:
- [ ] alle Bestimmungsstaaten
- [ ] alle Bestimmungsstaaten mit Ausnahme der Vereinigten Staaten von Amerika
- [X] nur die Vereinigten Staaten von Amerika
- [ ] die im Zusatzfeld angegebenen Staaten

Name und Anschrift: *(Familienname, Vorname: bei juristischen Personen vollständige amtliche Bezeichnung. Bei der Anschrift sind die Postleitzahl und der Name des Staats anzugeben)*

Diese Person ist:
- [ ] nur Anmelder
- [ ] Anmelder und Erfinder
- [ ] nur Erfinder *(Wird dieses Kästchen angekreuzt, so sind die nachstehenden Angaben nicht nötig.)*

Staatsangehörigkeit (Staat):

Sitz oder Wohnsitz (Staat):

Diese Person ist Anmelder für folgende Staaten:
- [ ] alle Bestimmungsstaaten
- [ ] alle Bestimmungsstaaten mit Ausnahme der Vereinigten Staaten von Amerika
- [ ] nur die Vereinigten Staaten von Amerika
- [ ] die im Zusatzfeld angegebenen Staaten

Name und Anschrift: *(Familienname, Vorname: bei juristischen Personen vollständige amtliche Bezeichnung. Bei der Anschrift sind die Postleitzahl und der Name des Staats anzugeben)*

Diese Person ist:
- [ ] nur Anmelder
- [ ] Anmelder und Erfinder
- [ ] nur Erfinder *(Wird dieses Kästchen angekreuzt, so sind die nachstehenden Angaben nicht nötig.)*

Staatsangehörigkeit (Staat):

Sitz oder Wohnsitz (Staat):

Diese Person ist Anmelder für folgende Staaten:
- [ ] alle Bestimmungsstaaten
- [ ] alle Bestimmungsstaaten mit Ausnahme der Vereinigten Staaten von Amerika
- [ ] nur die Vereinigten Staaten von Amerika
- [ ] die im Zusatzfeld angegebenen Staaten

Name und Anschrift: *(Familienname, Vorname: bei juristischen Personen vollständige amtliche Bezeichnung. Bei der Anschrift sind die Postleitzahl und der Name des Staats anzugeben)*

Diese Person ist:
- [ ] nur Anmelder
- [ ] Anmelder und Erfinder
- [ ] nur Erfinder *(Wird dieses Kästchen angekreuzt, so sind die nachstehenden Angaben nicht nötig.)*

Staatsangehörigkeit (Staat):

Sitz oder Wohnsitz (Staat):

Diese Person ist Anmelder für folgende Staaten:
- [ ] alle Bestimmungsstaaten
- [ ] alle Bestimmungsstaaten mit Ausnahme der Vereinigten Staaten von Amerika
- [ ] nur die Vereinigten Staaten von Amerika
- [ ] die im Zusatzfeld angegebenen Staaten

- [ ] Weitere Anmelder und/oder (weitere) Erfinder sind auf einem zusätzlichen Fortsetzungsblatt angegeben.

Feld Nr. V BESTIMMUNG VON STAATEN

Die folgenden Bestimmungen nach Regel 4.9 Absatz a werden hiermit vorgenommen *(bitte die entsprechenden Kästchen ankreuzen; wenigstens ein Kästchen muß angekreuzt werden):*

Regionales Patent

- [X] **AP** **ARIPO-Patent:** **KE** Kenia, **LS** Lesotho, **MW** Malawi, **SD** Sudan, **SZ** Swasiland, **UG** Uganda und jeder weitere Staat, der Vertragsstaat des Harare-Protokolls und des PCT ist
- [ ] **EA** **Eurasisches Patent:** **AM** Armenien, **AZ** Aserbaidschan, **BY** Belarus, **KG** Kirgisistan, **KZ** Kasachstan, **MD** Republik Moldau, **RU** Russische Föderation, **TJ** Tadschikistan, **TM** Turkmenistan und jeder weitere Staat, der Vertragsstaat des Eurasischen Patentübereinkommens und des PCT ist
- [X] **EP** **Europäisches Patent:** **AT** Österreich, **BE** Belgien, **CH und LI** Schweiz und Liechtenstein, **DE** Deutschland, **DK** Dänemark, **ES** Spanien, **FI** Finland, **FR** Frankreich, **GB** Vereinigtes Königreich, **GR** Griechenland, **IE** Irland, **IT** Italien, **LU** Luxemburg, **MC** Monaco, **NL** Niederlande, **PT** Portugal, **SE** Schweden und jeder weitere Staat, der Vertragsstaat des Europäischen Patentübereinkommens und des PCT ist
- [X] **OA** **OAPI-Patent:** **BF** Burkina Faso, **BJ** Benin, **CF** Zentralafrikanische Republik, **CG** Kongo, **CI** Côte d'Ivoire, **CM** Kamerun, **GA** Gabun, **GN** Guinea, **ML** Mali, **MR** Mauretanien, **NE** Niger, **SN** Senegal, **TD** Tschad, **TG** Togo und jeder weitere Staat, der Vertragsstaat der OAPI und des PCT ist *(falls eine andere Schutzrechtsart oder ein sonstiges Verfahren gewünscht wird, bitte auf der gepunkteten Linie angeben)* .....

Nationales Patent *(falls eine andere Schutzrechtsart oder ein sonstiges Verfahren gewünscht wird, bitte auf der gepunkteten Linie angeben):*

- [X] **AL** Albanien .....
- [X] **AM** Armenien .....
- [ ] **AT** Österreich .....
- [X] **AU** Australien .....
- [X] **AZ** Aserbaidschan
- [X] **BB** Barbados
- [X] **BG** Bulgarien .....
- [X] **BR** Brasilien .....
- [X] **BY** Belarus
- [X] **CA** Kanada
- [ ] **CH und LI** Schweiz und Liechtenstein
- [X] **CN** China .....
- [X] **CZ** Tschechische Republik .....
- [ ] **DE** Deutschland .....
- [ ] **DK** Dänemark .....
- [X] **EE** Estland .....
- [ ] **ES** Spanien .....
- [ ] **FI** Finnland .....
- [ ] **GB** Vereinigtes Königreich
- [X] **GE** Georgien .....
- [X] **HU** Ungarn .....
- [X] **IL** Israel
- [X] **IS** Island
- [X] **JP** Japan .....
- [ ] **KE** Kenia .....
- [X] **KG** Kirgisistan .....
- [X] **KP** Demokratische Volksrepublik Korea .....
- [X] **KR** Republik Korea .....
- [X] **KZ** Kasachstan .....
- [X] **LK** Sri Lanka
- [X] **LR** Liberia
- [ ] **LS** Lesotho .....
- [X] **LT** Litauen
- [ ] **LU** Luxemburg .....
- [X] **LV** Lettland
- [X] **MD** Republik Moldau .....
- [X] **MG** Madagaskar .....
- [X] **MK** Die ehemalige jugoslawische Republik Mazedonien .....
- [X] **MN** Mongolei
- [ ] **MW** Malawi .....
- [X] **MX** Mexiko .....
- [X] **NO** Norwegen
- [X] **NZ** Neuseeland .....
- [X] **PL** Polen .....
- [ ] **PT** Portugal .....
- [X] **RO** Rumänien
- [X] **RU** Russische Föderation .....
- [ ] **SD** Sudan
- [ ] **SE** Schweden
- [X] **SG** Singapur
- [X] **SI** Slowenien .....
- [X] **SK** Slowakei .....
- [X] **TJ** Tadschikistan .....
- [X] **TM** Turkmenistan .....
- [X] **TR** Türkei .....
- [X] **TT** Trinidad und Tobago .....
- [X] **UA** Ukraine .....
- [ ] **UG** Uganda .....
- [X] **US** Vereinigte Staaten von Amerika .....
- [X] **UZ** Usbekistan .....
- [X] **VN** Vietnam .....

Kästchen für die Bestimmung von Staaten (für die Zwecke eines nationalen Patents), die dem PCT nach der Veröffentlichung dieses Formblatts beigetreten sind:

- [X] **CU** Kuba .....
- [X] **LC** St. Lucia .....
- [X] **BA** Bosnien und Herzegowina .....
- [X] **YU** Jugoslawien

Zusätzlich zu den oben genannten Bestimmungen nimmt der Anmelder nach Regel 4.9 Absatz b auch alle anderen nach dem PCT zulässigen Bestimmungen vor mit Ausnahme der Bestimmung von _____.

Der Anmelder erklärt, daß diese zusätzlichen Bestimmungen unter dem Vorbehalt einer Bestätigung stehen und jede zusätzliche Bestimmung, die vor Ablauf von 15 Monaten ab dem Prioritätsdatum nicht bestätigt wurde, nach Ablauf dieser Frist als vom Anmelder zurückgenommen gilt. *(Die Bestätigung einer Bestimmung erfolgt durch die Einreichung einer Mitteilung, in der diese Bestimmung angegeben wird, und die Zahlung der Bestimmungs- und der Bestätigungsgebühr. Die Bestätigung muß beim Anmeldeamt innerhalb der Frist von 15 Monaten eingehen.)*

PCT/EP 97/01741

Feld Nr. VI PRIORITÄTSANSPRUCH Weitere Prioritätsansprüche sind im Zusatzfeld angegeben. ☐

Die Priorität der folgenden früheren Anmeldung(en) wird hiermit beansprucht:

Staat (Anmelde- oder Bestimmungsstaat der Anmeldung)	Anmeldedatum (Tag/Monat/Jahr)	Aktenzeichen	Anmeldeamt (nur bei regionaler oder internationaler Anmeldung)
(1) EP DE	(11.04.96) 11. April 1996	96 105 679.3	EP ~~EPA/München~~
(2)			
(3)			

Dieses Kästchen ankreuzen, wenn die beglaubigte Kopie der früheren Anmeldung von dem Amt ausgestellt werden soll, das für die Zwecke dieser internationalen Anmeldung Anmeldeamt ist (eine Gebühr kann verlangt werden):

☐ Das Anmeldeamt wird hiermit ersucht, eine beglaubigte Abschrift der oben in Zeile(n) _____ bezeichneten früheren Anmeldung(en) zu erstellen und dem Internationalen Büro zu übermitteln.

Feld Nr. VII INTERNATIONALE RECHERCHENBEHÖRDE

Wahl der Internationalen Recherchenbehörde (ISA) *(Sind zwei oder mehr Internationale Recherchenbehörden für die internationale Recherche zuständig, ist der Name der Behörde anzugeben, die die internationale Recherche durchführen soll; Zweibuchstaben-Code genügt):* ISA/_____

Frühere Recherche: *Auszufüllen, wenn eine Recherche (internationale Recherche, Recherche internationaler Art oder sonstige Recherche) bereits bei der internationalen Recherchenbehörde beantragt oder von ihr durchgeführt worden ist und diese Behörde nun ersucht wird, die internationale Recherche soweit wie möglich auf die Ergebnisse einer solchen früheren Recherche zu stützen. Die Recherche oder der Recherchenantrag ist durch Angabe der betreffenden Anmeldung (bzw. deren Übersetzung) oder des Recherchenantrags zu bezeichnen.*

Staat (oder regionales Amt):	Datum (Tag/Monat/Jahr):	Aktenzeichen:
EPA/Den Haag	04. November 1996	96 105 679.6

Feld Nr. VIII KONTROLLISTE

Diese internationale Anmeldung umfaßt:

1. Antrag	:	6 Blätter
2. Beschreibung	:	20 Blätter
3. Ansprüche	:	2 Blätter
4. Zusammenfassung	:	1 Blätter
5. Zeichnungen	:	Blätter
Insgesamt	:	29 Blätter

Dieser internationalen Anmeldung liegen die nachstehend angekreuzten Unterlagen bei:

1. ☐ Unterzeichnete gesonderte Vollmacht
2. ☐ Kopie der allgemeinen Vollmacht
3. ☐ Begründung für das Fehlen der Unterschrift
4. ☒ Prioritätsbeleg(e) *(durch die Zeilennummer von Feld Nr. VI kennzeichnen):*
5. ☒ Blatt für die Gebührenberechnung
6. ☐ Gesonderte Angaben zu hinterlegten Mikroorganismen
7. ☐ Sequenzprotokolle für Nucleotide und/oder Aminosäuren (Diskette)
8. ☐ Sonstige *(einzeln aufführen):*

Abbildung Nr. _____ der Zeichnungen (falls vorhanden) soll mit der Zusammenfassung veröffentlicht werden.

Feld Nr. IX UNTERSCHRIFT DES ANMELDERS ODER DES ANWALTS

Der Name jeder unterzeichnenden Person ist neben der Unterschrift zu wiederholen, und es ist anzugeben, sofern sich dies nicht eindeutig aus dem Antrag ergibt, in welcher Eigenschaft die Person unterzeichnet.

Hoechst Schering AgrEvo GmbH

W. Schmidt (Dr. Schmidt) Patente, Frankfurt

(Dr. Weißert) Patente, Frankfurt

Vom Anmeldeamt auszufüllen

1. Datum des tatsächlichen Eingangs dieser internationalen Anmeldung: 08 APR 1997 (08 APR. 1997)	2. Zeichnungen ☐ eingegangen: ☐ nicht eingegangen:
3. Geändertes Eingangsdatum aufgrund nachträglich, jedoch fristgerecht eingegangener Unterlagen oder Zeichnungen zur Vervollständigung dieser internationalen Anmeldung:	
4. Datum des fristgerechten Eingangs der angeforderten Richtigstellungen nach Artikel 11(2) PCT:	
5. Vom Anmelder benannte Internationale Recherchenbehörde: ISA/	6. ☐ Übermittlung des Recherchenexemplars bis zur Zahlung der Recherchengebühr aufgeschoben

Vom Internationalen Büro auszufüllen

Datum des Eingangs des Aktenexemplars beim Internationalen Büro:

Zusatzfeld *Wird dieses Zusatzfeld nicht benutzt, so ist dieses Blatt dem Antrag nicht beizufügen.*

Dieses Feld ist in folgenden Fällen auszufüllen:

1. Wenn der Platz in einem Feld nicht für alle Angaben ausreicht:	***In diesem Fall sind mit dem Vermerk "Fortsetzung von Feld Nr. ..." [Nummer des Feldes angeben] die gleichen Angaben zu machen wie in dem Feld vorgesehen, das platzmäßig nicht ausreicht:***
insbesondere:	
i) Wenn mehr als zwei Anmelder und/oder Erfinder vorhanden sind und kein Fortsetzungsblatt zur Verfügung steht:	*In diesem Fall sind mit dem Vermerk "Fortsetzung von Feld Nr. III" für jede weitere Person die in Feld Nr. III vorgesehenen Angaben zu machen.*
ii) Wenn in Feld Nr. II oder III die Angabe "die im Zusatzfeld angegebenen Staaten" angekreuzt ist:	*In diesem Fall sind mit dem Vermerk "Fortsetzung von Feld Nr. II", "Fortsetzung von Feld Nr. III" oder "Fortsetzung von Feld Nr. II und Nr. III" die Namen der Anmelder und neben jedem Namen der Staat oder die Staaten (und/oder ggf. ARIPO-, eurasisches, europäisches oder OAPI-Patent) anzugeben, für die die bezeichnete Person Anmelder ist.*
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Fortsetzung von Feld Nr. IX

1) Günter DONN

2) Peter ECKES

3) Hubert MÜLLNER

Zusatzfeld *Wird dieses Zusatzfeld nicht benutzt, so ist dieses Blatt dem Antrag nicht beizufügen.*

Dieses Feld ist in folgenden Fällen auszufüllen:

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vi) Wenn die Priorität von mehr als drei früheren Anmeldungen beansprucht wird:	*In diesem Fall sind mit dem Vermerk "Fortsetzung von Feld Nr. VI" für jede weitere frühere Anmeldung die gleichen Angaben zu machen wie in Feld Nr. VI vorgesehen.*
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Fortsetzung von Feld Nr. IX

4) Genes DUDITS

5) Katalin PAULOVICS

6) Attila FEHER

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(54) Title: PROCESS FOR THE PRODUCTION OF PLANTS WITH ENHANCED GROWTH CHARACTERISTICS

(57) Abstract

A process for the production of plants with improved growth characteristics by targeted expression of bacterial asparagines synthetase in the chloroplasts or plastids, and plants therefrom, are disclosed and claimed, together with intermediates therefor.

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TITLE OF THE INVENTION

Process for the production of plants with enhanced growth characteristics

RELATED APPLICATIONS

Reference is made to U.S. application Serial No. 08/465,526, filed June 5, 1995, as a division of U.S. application Serial No. 08/360,176, now U.S. Patent No. 5,545,819; each of these U.S. applications and U.S. Patent are hereby incorporated herein by reference.

FIELD OF THE INVENTION

The invention relates to: improving plant growth by expression of at least one bacterial asparagine synthetase in the chloroplast and/or plastid of cells of the plant; methods for so improving plant growth including introducing a nucleic acid molecule encoding the bacterial asparagine synthetase into the plant genome (e.g., into plant cells and culturing and/or regenerating the cells into the plants) wherein the nucleic acid molecule is operably linked to a nucleic acid molecule comprising regulatory sequences for expression and for import of the bacterial asparagine synthetase into the chloroplast and/or plastid; and, to plants having such improved growth.

Several documents are cited in the following text. Documents cited herein are hereby incorporated herein by reference.

BACKGROUND OF THE INVENTION

Nitrogen often is the rate-limiting element in plant growth. Most field crops have a fundamental dependence on inorganic nitrogenous fertilizer. Mineral fertilizers are a major source for ground water pollution. Therefore it would be beneficial if plants could utilize the existing nitrogen more efficiently.

Nitrogen is taken up by the plant as inorganic compounds, namely nitrate and ammonia. The majority of this nitrogen is assimilated into organic compounds like amino acids. The enzyme glutamine sythetase plays a major role since it catalyses the assimilation of ammonia into glutamine. Glutamine together with asparagines are the main transport forms of nitrogen in plants. As described in EP 511 979 the expression of a bacterial asparagines synthetases leads to improved growth characteristics which may be enhanced by the additional treatment of the plants with the herbicide glufosinate, a glutamine synthetase inhibitor. Whereas WO 95/09911 describes the production of a plant with improved agronomic or nutritional characteristics by over expression of one or several nitrogen/metabolism enzymes Applicants have now been able to find a quite different way to improve plant growth characteristics.

SUMMARY OF THE INVENTION

It has surprisingly be found that it is possible to improve plant growth capacities by the targeted expression of at least one bacterial asparagine synthetase in the chloroplast.

The present invention is directed to a process for the production of plants with improved growth characteristics which comprises the following steps:

- transfer and integration of a DNA sequence coding for a bacterial asparagine synthetases in the plant genome
- wherein said DNA sequence is linked to regulatory sequences which ensures expression of said gene in a plant cell and leading to the import of the derived protein into the chloroplast and/or plastids of said plant cells and
- regeneration of intact and fertile plants from the transformed cells.

According to instant invention the term improved growth characteristics is to be understood as encompassing enhanced or faster and more vigorous growth as well as more yield and/or earlier flowering. The process according to instant invention leads also to bigger or more reproductive organs as for example the seeds or bigger or more storage organs as for example tubers.

According to instant invention the bacterial asparagines synthetases may also be expressed directly in the chloroplast by integrating the gene directly into the genome of the chloroplast and/or plastids by for example the biolistic transformation procedure (see US Patent No. 5,451,513 incorporated herein by reference).

Therefore, the instant invention is also directed to a process for the production of plants with improved growth characteristics which comprises the following steps:

- transfer and integration of a DNA sequence coding for a bacterial asparagine synthetases into the genome of the chloroplast and/or plastids of a plant

cells,

- expression of said gene under the control of appropriate regulatory elements and
- regeneration of intact and fertile plants from the transformed cells.

Surprisingly, it was possible to enhance the growth improving effect even more by reducing the level of the glutamine synthetase expressed in the plant cell.

Accordingly, the instant invention is also directed to processes for the production of plant cells wherein said plant cells express a further gene construct which leads to a reduced level of its endogeneous glutamine synthetase activity.

A "DNA sequence", as the term is used herein, can mean a nucleic acid molecule, e.g., an isolated nucleic acid molecule; and, a "regulatory sequence", as the term is used herein, can mean a nucleic acid molecule which functions to regulate expression and/or import, e.g., import into a chloroplast and/or plastid.

Thus, the invention provides a plant cell containing DNA coding for prokaryotic, e.g., bacterial, asparagine synthetase, e.g., ammonium-specific asparagine synthetase, type A, operably linked to a regulatory sequence for expression of the DNA and import of the asparagine synthetase into the chloroplast and/or plastid of the cell, wherein the cell expresses the asparagine synthetase. Thus, the plant cell expresses the asparagine synthetase in its chloroplast and/or plastid. The plant cell can also contain a construct which provides reduced levels of expression of

endogenous glutamine synthetase, e.g., the endogenous gene therefor can be deleted or disrupted.

The invention further provides a method for increasing growth of a plant comprising: transforming a plant cell so that the cell contains DNA coding for prokaryotic, e.g., bacterial asparagine synthetase, e.g., ammonium-specific asparagine synthetase, type A, operably linked to a regulatory sequence for expression of the DNA and import of the asparagine synthetase into the chloroplast and/or plastid of the cell, wherein the cell expresses the asparagine synthetase (e.g., in its chloroplast and/or plastid); and regenerating the plant from the cell. The plant is preferably intact and fertile.

The plant cell in the method can also have the endogenous gene for glutamine synthetase deleted or disrupted, or otherwise expressed at a reduced level. Thus, the method can include transforming a plant cell to have a reduced level of expression of endogenous glutamine synthetase (e.g., by disrupting or deleting the gene therefor) and so that the cell contains DNA coding for prokaryotic, e.g., bacterial asparagine synthetase, e.g., ammonium-specific asparagine synthetase, type A, operably linked to a regulatory sequence for expression of the DNA and import of the asparagine synthetase into the chloroplast and/or plastid of the cell, wherein the cell expresses the asparagine synthetase (e.g., in its chloroplast and/or plastid); and regenerating the plant from the cell. The plant is preferably intact and fertile.

The methods can further comprise treating the plant with a glutamine synthetase

inhibitor.

The DNA coding for the asparagine synthetase can be from *E. coli*. However, from this disclosure, and the documents cited herein, and the knowledge in the art, one skilled in the art can ascertain other genes encoding asparagine synthetase, i.e., asn-A genes, from other microorganisms, e.g. by any routine procedure, for instance:

1. Ascertaining an asn-A gene product activity by routine assays for the asparagine synthetase type A with subsequent purification of the enzyme, e.g., according to Cedar & Schwartz 1969, J. Biol. Chem., 244, 4112-21 and 4122-4127, Humbert & Simoni, 1980, J. Bacteriol., 142, 212-220, and Reitzer & Magasanik, 1982, J. Bacteriol., 151, 1299-1313; see also Herrmann and Somerville, "Amino Acids, Biosynthesis And Genetic Regulation", pp. 137-145 (Addison-Wesley Pub. Co. 1993).
2. Production and purification of polyclonal antibodies against the asn-A gene product according to well-known immunological methods. And,
3. Screening of expression libraries of microorganisms with isolated antibodies against asparagine synthetase type A according to well-known molecular biological methods.

The above-described procedures make it clear that a skilled artisan can obtain asn-A gene sequences from other microorganisms by routine methods. Preferred asparagine synthetase utilizes ammonium ions as an amide donor for the production of asparagine; and thus, preferred DNA encodes such asparagine synthetase. Further, the regulatory sequence can be for a chloroplastic leader peptide; and, the

DNA coding for asparagine synthetase and the regulatory sequence can thus encode a prokaryotic asparagine synthetase, e.g., a bacterial asparagine synthetase such as *E. coli* asparagine synthetase, with a chloroplastic peptide at its N-terminal.

In the methods described herein, the growth of the plant is increased relative to non-transformed plants.

The invention further comprehends a plant, seeds, propagule or propagation material, from the foregoing methods, or containing the foregoing cells.

Additionally, the invention comprehends a gene construct comprising an isolated nucleic acid molecule encoding a prokaryotic, e.g., bacterial, asparagine synthetase, e.g., ammonium-specific asparagine synthetase, type A, operatively linked to a regulatory sequence active in plants for expression of the nucleic acid molecule and import of the asparagine synthetase into the chloroplast and/or plastid of cells of plants, e.g., a chloroplastic leader peptide; and therefore, in an embodiment the invention can provide a gene construct comprising an isolated nucleic acid molecule encoding a prokaryotic, e.g., bacterial such as *E. coli*, asparagine synthetase with a chloroplastic leader at its N-terminus. The invention also comprehends vectors containing the inventive gene constructs. The vector can be useful for transforming plant cells. Thus, the invention comprehends a plant cell transformed with the gene construct or vector, as well as plants, seeds, and propagules or propagation materials containing such cells.

And, the invention comprehends gene constructs and vectors for reducing endogenous glutamine synthetase expression, e.g., for inserting termination codons after regulatory sequences and prior to coding sequences, or for otherwise disrupting the gene for endogenous glutamine synthetase, as well as cells transformed with such gene constructs or vectors, and plants, seeds and propagules or propagation materials containing such cells.

These and other embodiments are disclosed or are obvious from and encompassed by, the following Detailed Description.

DETAILED DESCRIPTION

A preferred method of introducing the nucleic acid segments into plant cells is to infect plant cells with A. tumefacient carrying an inserted DNA construct. The nucleic acid segments or constructs can be introduced into appropriate plant cells, for example, by means of the Ti plasmid of A. tumefaciens. The T-DNA is transmitted to plant cells upon infection by A. tumefaciens, and is stably integrated into the plant genome. Under appropriate conditions known in the art, the transformed cells develop further into plants.

The Agrobacterium strains customarily employed in the art of transformation are described, for example see especially US Patent No. 5,188,958 and EP 0 270 615 B1, incorporated herein by reference.

Ti plasmids contain two regions essential for the production of transformed cells. One of these, named transfer DNA (T DNA), induces tumour formation. The other,

termed virulent region, is essential for the introduction of the T DNA into plants. The transfer DNA region, which is transferred into the plant genome, can be increased in size by the insertion of the foreign nucleic acid sequence without its ability of transfer being affected. By removing the tumour-causing genes so that they no longer interfere the modified Ti plasmid ("disarmed Ti vector") can then be used as a vector for the transfer of the gene constructs of the invention into an appropriate microspores. In the binary system, to have infection, two plasmids are needed: a T-DNA containing plasmid and a vir plasmid (see especially EP 116718 B1 and EP 120 516 B1).

Besides transformation using Agrobacteria there are many other techniques for the introduction of DNA available. These techniques include, e.g. the protoplast transformation (see EP 164 575) the micro injection of DNA, the introduction of DNA via electroporation as well as biolistic methods and virus mediated infection. From the transformed cells applying suitable media and techniques whole plants can be regenerated (see McCormick et al. (1986) in Plant Cell Reports 5: 81-84). The regenerated plants may be preferably used to cross them with existing breeding lines to improve their growth characteristics as well.

The DNA constructs used in instant invention consist of a transcription initiation region and, under the control of the transcription initiation region, a DNA sequence to be transcribed. The DNA sequence may comprise a natural open reading frame including transcribed 5' and 3' flanking sequences. Alternatively, it may comprise an anti-sense sequence that encodes the complement of an RNA molecule or portion thereof (as described in EP 140 308 B1 and EP 223 399 B1) in order to suppress

the expression of the internally expressed glutamine synthetases.

The initiation regions may be used in a variety of contexts and in combination with a variety of sequences. The RNA coded sequences of a gene may be those of a natural gene, including the open reading frame for protein coding and frequently the 5' and 3' untranslated sequences. The RNA translational initiation sequences are included in the constructs, either from the promoter domain or from the attached coding sequences.

Attached to the above sequences are appropriate transcription termination and polyadenylation sequences.

The DNA constructs used in the transformation process according to instant invention may comprise sequences coding for naturally occurring or genetically modified transit peptides (see for example EP 189 707 B1).

Examples of additionally expressed sequences or genes to be expressed from the constructs of the subject invention include:

- especially antisense or sense genes (for gene suppression or cosuppression); as well as additionally
- nutritionally important proteins: growth promoting factors;
- yield enhancing genes or factors, e.g. an invertase gene, a citrate synthase, a polyphosphate kinase;
- proteins giving protection to the plant under certain environmental conditions, e.

g. proteins giving resistance to metal or other toxicity;

- stress related proteins giving tolerance to extremes of temperature, freezing, etc.
- proteins of specific commercial value;
- genes causing increased level of proteins, e. g., enzymes of metabolic pathways,
- genes causing increased levels of products of structural value to a plant host, e. g., herbicide resistance, fungus resistance, e.g. chitinase genes, glucanase genes, proteins synthesis inhibitor genes, ribosome inhibitory protein genes, viral resistance, e.g. ribozymes, virus coat protein genes.

The subject constructs will be prepared employing cloning vectors, where the sequences may be naturally occurring, mutated sequences, synthetic sequences, or combinations thereof. The cloning vectors are well known and comprise prokaryotic replication systems, markers for selection of transformed host cells, and restriction sites for insertion or substitution of sequences. For transcription and optimal expression, the DNA may be transformed into plant cells for integration into the genome, where the subject construct is joined to a marker for selection or is co-transformed with DNA encoding a marker for selection.

The selection of transformed cells is enabled by the use of a selectable marker gene which is also transferred. The expression of the marker gene confers a phenotypic trait that enables the selection. Examples for such genes are those coding for antibiotics or herbicide resistance, e.g. genes causing resistance against glutamine synthetases inhibitors, e.g. bialaphos or phosphinothricin resistance conferred by genes isolated from Streptomyces hygroscopicus or viridochromogenes (BAR/PAT). Other examples are the neomycin phosphotransferase or the glucuronidase gene.

The class of transgenic plants which are covered by this invention is generally as broad as the class of higher plants susceptible to transformation, including both monocotyledonous and dicotyledonous plants. It is known that theoretically all plants can be regenerated from cultured totipotent cells, including but not limited to all major cereal crop species, sugarcane, sugar beet, cotton, fruit and other trees, legumes and vegetables.

Examples of families that are of special interest are Poaceae, but also Solanaceae, Malvaceae and Brassicaceae.

Some suitable species include, for example, species from the genera Fragaria, Lotus, Medicago, Onobrychis, Trifolium, Trigonella, Vigna, Citrus, Linum, Geranium, Manihot, Daucus, Arabidopsis, Brassica, Raphanus, Sinapis, Atropa, Capsicum, Hyoscyamus, Lycopersicon, Nicotiana, Solanum, Petunia, Digitalis, Majorana, Ciohorium, Helianthus, Lactuca, Bromus, Asparagus, Antirrhinum, Hererocallis, Nemesia, Pelargonium, Panicum, Pennisetum, Ranunculus, Senecio, Salpiglossis, Cucumis, Browaalia, Glycine, Lolium, Zea, Triticum, Sorghum, and Datura.

Examples of species of commercial interest that can be protected include:

- tobacco, Nicotiana tabacum L.
- tomato, Lycopersicon esculentum Mill,
- potato, Solanum tuberosum L.,
- Canola/Rapeseed,
- Brassica napus L.,

- cabbage, broccoli, kale etc.,
- Brassica oleracea L.,
- mustards Brassica juncea L.,
- Brassica nigra L.,
- Sinapis alba L. (Brassicaceae),
- petunia, Petunia hybrida (Solanaceae)
- sugar beet, Beta vulgaris,(Chenopodiaceae),
- cucumber, Curcurbita sp. (Curcurbitaceae),
- cotton, Gossypium sp., (Malvaceae),
- sunflower, Helianthus annuus,
- lettuce Lactuca sativa, (Asteraceae=Compositae),
- pea, Pisum sativum,
- soybean, Glycine max and alfalfa, Medicago sp. (Fabaceae=Leguminoseae),
- asparagus, Asparagus officinalis;
- gladiolus, Gladiolus sp., (Lilaceae);
- corn, Zea mays;
- rice, Oryza sativa (Poaceae);
- wheat, Triticum aestivum (Poaceae); and
- barley, Hordeum vulgare (Poaceae).

In an preferred embodiment the invention covers transformed potato, tobacco, corn, sugar beet, cotton, rape seed, soy bean, lupine, rice and wheat. Especially preferred are potatoes

The invention additionally relates to transformed plants which have been

regenerated out of different cell types and which have been transformed according to instant invention.

The transformation can be carried out as described in the following examples, provicded by way of illustration only.

EXAMPLES

In general, preparation of plasmid DNA, restriction enzyme digestion, agarose gel electrophoresis of DNA, Southern blots, DNA ligation and bacterial transformation were carried out using standard methods. (Maniatis et al., Molecular Cloning, a Laboratory Manual, Cold Spring Harbor Laboratory (1982), referred to herein as "Maniatis" and hereby incorporated by reference.)

Example 1:

Fusion of a bacterial asparagine synthetase gene to the nucleotide sequence for a duplicated chloroplast transit peptide

Based on the complete nucleotide sequence of the ASN-A gene from E. coli (Nakamura et al. (1981) or EP 511 979) the gene was cloned as a Hga 1 /Pst 1 fragment into the vector pUC18. By means of PCR based in vitro mutagenesis a SphI site was created at the ATG translational start codon changing the nucleotide sequence from AAA ATG AAA ACC GCT (SEQ ID No: 1) into GGC GCATG CAG AAA ACC GCT (SEQ ID No.: 2). This mutation introduced an additional codon for glutamic acid into the gene directly following the ATG translation start codon.

The nucleotide sequence for a modified transit peptide from the small subunit of Ribulosebisphosphat Carboxylase from pea was isolated from the vector pNi6/25 (Wasmann, C.C. et al (1986) Mol. Gen. Genet. 205: 446-453) as a Hind3/Sph1 fragment. This transit peptide contains a duplication of 20 amino acids compared to the natural transit peptide.

The sequence of the duplicated transit peptide and ASN-A gene were fused by ligating the Sph1 sites resulting in tpASN. The tpASN gene was exised as a Hind3/Pst1 fragment and after changing the Hind3 site into a Kpn1 site cloned between CaMV 35S promoter and -terminator of the vector pDH51 $^{\delta}$Kpn.

Example 2:

Expression of the tpASN gene in tobacco and rape seed

The 35S-promoter/tpASN gene/35S-terminator cassette from pDH51 $^{\delta}$Kpn was isolated as an EcoR1 fragment, Hind3 linkers were added and the fragment was cloned into the Hind3 site of the vector pHOE6/Ac, which confers phosphinothricin resistance to plants. The resulting vector was called pHOE6Ac/tpASN. This vector was transformed into the C58 Agrobacterium strain MP90RK (Koncz et al., Mol. Gen. Gen., 204, 383-396 (1986)).

Tobacco and rape seed plants were transformed following published procedures. Plants were regenerated on Murashige and Skoog based media.

Transformed plants were selected because of their resistance to the herbicide

phosphinothricin (PPT). PPT resistant plants were analysed for the presence of the bacterial asparagine synthetase gene. In a Northern Blot analysis ASN-A specific RNA was detected in the plants. With polyclonal antibodies it is demonstrated that the protein was targeted into the chloroplasts.

Example 3:

Expression of the tpASN gene in maize

The 35S-promoter/tpASN gene/35S-terminator cassette from pDH51 δKpn was isolated as an EcoR1 fragment, Hind3 linkers were added and the fragment was cloned into the Hind3 site of the vector pB2/35SAc resulting in pB35SAc/tpASN. This vector was used to transform maize protoplasts according to published procedures (EP 511 979 or EP 164 575). Plants were regenerated on Murashige and Skoog based media. Transformed plants were selected because of their resistance to the herbicide phosphinothricin (PPT). PPT resistant plants were analysed for the presence of the bacterial asparagine synthetase gene. In a Northern Blot analysis ASN-A specific RNA was detected in the plants. With polyclonal antibodies it is demonstrated that the protein was targeted into the chloroplasts.

Example 4:

Inhibition of chloroplastic glutamine synthetase by expression of the antisense gene in tobacco and rape seed

The coding sequences for the chloroplastic isoenzymes of Nicotiana sylvestris and

Brassica napus were cloned by PCR methods from the genomic DNA of the respective plants. The resulting fragments were cloned as ApaI fragments in antisense orientation between 35S-promoter and -terminator from CaMV located on the vector pRT100. The 35S-promoter/GS-antisense/35S-terminator cassettes were isolated as Pst1 fragments and cloned into the Pst1 site of the vector pHOE6/AcK3. This vector was transformed into the C58 Agrobacterium strain MP9ORK (Koncz et al. supra (1986)). Tobacco and rape seed plants were transformed following published procedures. Plants were regenerated on Murashge and Skoog based media with reduced amounts of ammonia as described.

Transformed plants were selected because of their resistance to the herbicide phosphinothricin (PPT). PPT resistant plants were screened with Southern Blot hybridization for the presence of the ASN-A gene. Southern positive plants were analysed for the inactivation of the chloroplastic glutamine synthetase gene by Northern blots. Plants with the most reduced GS RNA level were selected.

Example 5:

Inhibition of chloroplastic glutamine synthetase by expression of the respective antisense gene in maize

The coding sequences for the chloroplastic isoenzymes of Zea mays, was cloned by PCR methods from the genomic DNA. The resulting fragment was cloned as ApaI fragment in antisense orientation between 35S-promoter and terminator from CaMV located on the vector pRT1OO. The 35S-promoter/GS-antisense/35S-terminator cassette was isolated as Pst1 fragment and cloned into the vector pB2/AcK3.

This vector was used to transform maize protoplasts according to published procedures. Plants were regenerated on Murashge and Skoog based media with reduced amounts of ammonia as described. Transformed plants were selected because of their resistance to the herbicide phosphinothricin (PPT). PPT resistant plants were screened with Southern Blot hybridization for the presence of the ASN-A gene. Southern positive plants were analysed for the inactivation of the chloroplastic glutamine synthetase gene by Northern blots. Plants with the most reduced GS RNA level were selected.

Example 6:

Asparagin content in transgenic asparagin synthetase expressing plants

Leaf material from wild type and different ransgenic asparagin synthetase expressing plants was homogenized in buffer. The extracts were run over a Biotronic amino acid analyser. Concentration of the amino acid asparagine were measured and are given in pmol/µl of extract.

	NT-WT	NT-TPASN-2	NT-TPASN-3	NT-TPASN-5	NT-TPASN-11
ASN	586,855	890,26	3338,5551	1506,6314	992,0319

The concentration of asparagine correlated with the expression of the asparagine synthetase gene as measured on Northern and Western Blots.

Example 7:

Production of transgenic potato lines carrying the bacterial asparagine synthetase gene

The above mentioned construct was used to transform potato plants (Solanum tuberosum L. cv. Desiree 25). The control, non-transformed plant material went through an in vitro regeneration process comparable to the transformants. The tuber tissues were transformed according to the process as described above using the Agrobacterium technology.

The presence of the bacterial asnA gene was proven by hybridization of genomic plant DNAs with a chimeric gene specific fragment. The experiments confirmed that the transformants expressed the transferred gene while the control plants lacked the enzyme.

Northern analysis was carried out by hybridization of total RNA from the transformed potato lines, the hybridization experiment indicated the presence of specific mRNA in the transformants whereas the control plant lines showed again no detectable signal.

Example 8:

Growth behaviour of transgenic maize and tobacco plants

Transgenic asparagine synthetase expressing plants and transgenic asparagine synthetase expressing plants with reduced glutamine synthetase activity were grown

side by side with wild type plants in the greenhouse. The transgenic plants showed a more vigorous growth and flowered earlier than wild type plants.

Field experiments with transgenic potato plants carrying the bacterial asparagine synthetase gene

Experiment A

Genotype	Tuber weight per plant (gram)	% of control
Control plant	135.0	100.0
Trans. Asl	168.6	124.0
Trans.As2	182.3	135.0

Experiment B

Genotype	Tuber weight per plot (kg)	% of control
Control Plant	8.16	100.0
Trans. Asl	11.39	139.5
Trans. As2	10.94	127.0

Having thus described in detail preferred embodiments of the present invention, it is to be understood that the invention defined by the appended claims is not to be limited to particular details set forth in the above description as many apparent variations thereof are possible without departing from the spirit or scope of the present invention.

WHAT IS CLAIMED IS:

1. A process for the production of plants with improved growth characteristics which comprises the following steps:
   - transfer and integration of a DNA sequence coding for a bacterial asparagine synthetase in the plant genome
   - wherein said DNA sequence is linked to a regulatory sequence which ensures expression of said gene in a plant cell and leading to the import of the derived protein into the chloroplasts and/or plastids of said plant cells and
   - regeneration of intact and fertile plants from the transformed cells.

2. A plant cell expressing a prokaryotic ammonium specific asparagine synthetase in its chloroplasts and plastids.

3. A plant cell according to claim 2 expressing further a gene construct leading to reduced level of its endogenous glutamine synthetase activity.

4. A plant, seeds and propagation material containing cells as claimed in claims 2 and 3.

5. A gene construct comprising a gene encoding a prokaryotic ammonium specific asparagine synthetase operatively linked to a regulatory sequence which ensures expression of said gene in a plant cell and leading to the import of the derived protein into chloroplasts and/or plastids of said plant cell.

6. A gene construct according to claim 5, wherein the asparagine synthetase gene is an E.coli asparagine Synthetase gene with a chloroplastic leader peptide at its N-terminus.

7. A vector containing a gene construct according to claims 5 and 6.

8. A plant cell transformed with the gene construct according to claim 5 and 6 or with a vector according to claim 7.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 97/01741

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/82 C12N15/52

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 95 09911 A (UNIV NEW YORK) 13 April 1995 cited in the application see page 22, line 1 - line 34 see page 28, line 1 - line 14 see page 65, line 21 - page 80, line 3 ---	1-8
Y	WO 91 11524 A (BIOLOG RESEARCH CENTRE ;HOECHST AG (DE)) 8 August 1991 cited in the application see the whole document ---	1-8
A	DD 288 618 A (AKADEMIE DER LANDWIRTSSCHAFTSWISSENSCHAFT DER DDR) 4 April 1991 see the whole document --- -/--	3

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
4 August 1997	08.08.97

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Maddox, A

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 97/01741

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	MOLECULAR AND GENERAL GENETICS, vol. 236, 1993, pages 315-325, XP002016640 TEMPLE, S.J., ET AL.: "Modulation of glutamine synthetase gene expression in tobacco by the introduction of an alfalfa glutamine synthetase gene in sense and antisense orientation: molecular and biochemical analysis" see the whole document	3
A	EP 0 508 909 A (RHONE POULENC AGROCHIMIE) 14 October 1992 see the whole document	1-8

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 97/01741

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9509911 A	13-04-95	AU 7928794 A	01-05-95
		CA 2173730 A	13-04-95
		DE 722494 T	03-04-97
		EP 0722494 A	24-07-96
		ES 2093578 T	01-01-97
WO 9111524 A	08-08-91	AU 7176891 A	21-08-91
		CN 1053641 A	07-08-91
		CS 9100165 A	15-09-91
		DE 69103404 D	15-09-94
		DE 69103404 T	28-09-95
		EP 0511979 A	11-11-92
		HU 65648 A	28-07-94
		TR 25404 A	01-03-93
		US 5545819 A	13-08-96
DD 288618 A		NONE	
EP 0508909 A	14-10-92	FR 2673643 A	11-09-92
		AU 652610 B	01-09-94
		AU 1144292 A	10-09-92
		CA 2061636 A	06-09-92
		IL 101115 A	10-01-97
		JP 5095789 A	20-04-93
		US 5510471 A	23-04-96
		US 5633448 A	27-05-97

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (PCT/IPEA/416)
1996/M206		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP97/01741	08/04/1997	11/04/1996
International Patent Classification (IPC) or national classification and IPC		
C12N15/52		
Applicant		
HOECHST SCHERING AGREVO GmbH et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 4 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 2 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
25/09/1997	14.07.98
Name and mailing address of the IPEA/	*Authorized officer*
European Patent Office D-80298 Munich Tel. (+49-89) 2399-0, Tx: 523656 epmu d Fax: (+49-89) 2399-4465	Merlos-Lange, A.M. Telephone No. (+49-89) 2399-8559

Form PCT/IPEA/409 (Cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP97/01741

I. Basis of the r port

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-20	as originally filed				

Claims, No.:

1-8	as received on	12/05/1998	with letter of	07/05/1998	

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-8
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-8
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-8
	No:	Claims	

2. Citations and explanations

see separate sheet

The amended set of claims 1-8 is allowable with respect to Art. 34 (2) PCT and appears to be novel in view of the available prior art (Art. 33(2) PCT).
It would further appear that the subject-matter claimed is also based on inventive activity in particular with respect to documents WO 95/09911 and WO 91/11542 which are considered to represent the closest prior art.
WO 95/09911 describes the production of plants with enhanced nitrogen assimilation, for example by suppressing the level of asparagine syntethase and/or glutamine synthetase or by ectopically overexpressing e.g. a eukaryotic asparagine synthetase.
WO 91/11524 discloses the expression of a prokaryotic ammonium specific asparagine synthetase in a plant previously transformed with the corresponding gene. In both cases plants with improved growth were obtained.
The problem to be solved by the present application is the further improvement of plant growth. This is solved by the targeted expression of an ammonium specific prokaryotic asparagine synthetase in particular in the chloroplasts, i.e. the prokaryotic gene is inter alia linked to regulatory sequences for import into the chloroplasts. This distinct and particular feature was not derivable from the above cited documents in an obvious manner.
The claims therefore appear to be in conformity with the requirements of Art. 33(3) PCT.

Replaced by Article 34

ANNEX

~~Substitute page~~

CLAIMS

1. A process for the production of plants with improved growth characteristics which comprises following steps:

- transfer and integration of a DNA sequence coding for a prokaryotic asparagine synthetase in the plant genome
- wherein said DNA sequence is linked to a regulatory sequence for the expression of said DNA and import of the asparagine synthetase into the chloroplasts and/or plastids of a plant cell and wherein said plant cell expresses the asparagine synthetase in its chloroplasts and/or plastids and
- regeneration of intact and fertile plants from the transformed cells.

2. A plant cell wherein a prokaryotic ammonium specific asparagine synthetase is expressed in its chloroplasts and plastids.

3. A plant cell according to claim 2 which contains a gene construct which provides a reduced level of expression of endogenous glutamine synthetase activity.

4. A plant, seeds and propagation material containing cells as claimed in claims 2 and 3.

5. A gene construct comprising a gene encoding a prokaryotic ammonium specific asparagine synthestase operatively linked to a regulatory sequence for the expression of said DNA and import of the asparagine synthetase into the chloroplasts and/or plastids of a plant cell and wherein said plant cell expresses the asparagine synthetase in its chloroplasts and/or plastids.

AMENDED SHEET

Substitute page

6. A gene construct according to claim 5, wherein the asparagine synthetase gene is an E. coli asparagine Synthetase gene with a chloroplastic leader peptide at its N-terminus.

7. A vector containing a gene construct according to claims 5 and 6 which gene construct comprises a sequence which encodes a chloroplastic leader peptide at its N-terminus.

8. A plant cell transformed with the gene construct according to claims 5 and 6 or with vector according to claim 7.

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

HOECHST SCHERING AGREVO GMBH
Patent- und Lizenzabteilung
Gebäude K 801
D-65926 Frankfurt am Main
ALLEMAGNE

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Rule 71.1)

Date of mailing *(day/month/year)*	14.07.98
Applicant's or agent's file reference: 1996/M206	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP97/01741	08/04/1997	11/04/1996

Applicant: HOECHST SCHERING AGREVO GmbH et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Hoechst Schering AgrEvo GmbH
Patent- u. Lizenzabteilung K 801
Vorg.
Eing. 16. JULI 1998

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16.7.98

Fr. Ripperger 17.7.98

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (PCT/IPEA/416)
1996/M206		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP97/01741	08/04/1997	11/04/1996
International Patent Classification (IPC) or national classification and IPC		
C12N15/52		
Applicant		
HOECHST SCHERING AGREVO GmbH et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 4 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 2 sheets.

3. This report contains indications relating to the following items:

I	☒	Basis of the report
II	☐	Priority
III	☐	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	☐	Lack of unity of invention
V	☒	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	☐	Certain documents cited
VII	☐	Certain defects in the international application
VIII	☐	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
25/09/1997	14.07.98
Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. (+49-89) 2399-0, Tx: 523656 epmu d Fax: (+49-89) 2399-4465	Merlos-Lange, A.M. Telephone No. (+49-89) 2399-8559

Form PCT/IPEA/409 (Cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP97/01741

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-20 as originally filed

Claims, No.:

1-8 as received on 12/05/1998 with letter of 07/05/1998

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-8
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-8
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-8
	No:	Claims	

2. Citations and explanations

see separate sheet

The amended set of claims 1-8 is allowable with respect to Art. 34 (2) PCT and appears to be novel in view of the available prior art (Art. 33(2) PCT).
It would further appear that the subject-matter claimed is also based on inventive activity in particular with respect to documents WO 95/09911 and WO 91/11542 which are considered to represent the closest prior art.
WO 95/09911 describes the production of plants with enhanced nitrogen assimilation, for example by suppressing the level of asparagine syntethase and/or glutamine synthetase or by ectopically overexpressing e.g. a eukaryotic asparagine synthetase.
WO 91/11524 discloses the expression of a prokaryotic ammonium specific asparagine synthetase in a plant previously transformed with the corresponding gene. In both cases plants with improved growth were obtained.
The problem to be solved by the present application is the further improvement of plant growth. This is solved by the targeted expression of an ammonium specific prokaryotic asparagine synthetase in particular in the chloroplasts, i.e. the prokaryotic gene is inter alia linked to regulatory sequences for import into the chloroplasts. This distinct and particular feature was not derivable from the above cited documents in an obvious manner.
The claims therefore appear to be in conformity with the requirements of Art. 33(3) PCT.

INTERNATIONAL SEARCH REPORT

[redacted] Application No
PCT/EP 97/01741

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/82 C12N15/52

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 95 09911 A (UNIV NEW YORK) 13 April 1995 cited in the application see page 22, line 1 - line 34 see page 28, line 1 - line 14 see page 65, line 21 - page 80, line 3 ---	1-8
Y	WO 91 11524 A (BIOLOG RESEARCH CENTRE ;HOECHST AG (DE)) 8 August 1991 cited in the application see the whole document ---	1-8
A	DD 288 618 A (AKADEMIE DER LANDWIRTSSCHAFTSWISSENSCHAFT DER DDR) 4 April 1991 see the whole document --- -/--	3

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
4 August 1997	08.08.97
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Authorized officer Maddox, A

Form PCT/ISA/210 (second sheet) (July 1992)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
1996/M206		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/EP 97/01741	08/04/1997	11/04/1996

Applicant

HOECHST SCHERING AGREVO GmbH et al.

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. [ ] **Certain claims were found unsearchable** (see Box I).

2. [ ] **Unity of invention is lacking** (see Box II).

3. [X] The international application contains disclosure of a **nucleotide and/or amino acid sequence listing** and the international search was carried out on the basis of the sequence listing
   - [ ] filed with the international application.
   - [X] furnished by the applicant separately from the international application,
     - [ ] but not accompanied by a statement to the effect that it did not include matter going beyond the disclosure in the international application as filed.
   - [ ] Transcribed by this Authority

4. With regard to the **title,**
   - [X] the text is approved as submitted by the applicant.
   - [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**
   - [X] the text is approved as submitted by the applicant.
   - [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this International Search Report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is:

   Figure No. _____
   - [ ] as suggested by the applicant.
   - [ ] because the applicant failed to suggest a figure.
   - [ ] because this figure better characterizes the invention.

   [X] None of the figures.

INTERNATIONAL SEARCH REPORT

[illegible] on patent family members

[illegible] Application No
PCT/EP 97/01741

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9509911 A	13-04-95	AU 7928794 A	01-05-95
		CA 2173730 A	13-04-95
		DE 722494 T	03-04-97
		EP 0722494 A	24-07-96
		ES 2093578 T	01-01-97
WO 9111524 A	08-08-91	AU 7176891 A	21-08-91
		CN 1053641 A	07-08-91
		CS 9100165 A	15-09-91
		DE 69103404 D	15-09-94
		DE 69103404 T	28-09-95
		EP 0511979 A	11-11-92
		HU 65648 A	28-07-94
		TR 25404 A	01-03-93
		US 5545819 A	13-08-96
DD 288618 A		NONE	
EP 0508909 A	14-10-92	FR 2673643 A	11-09-92
		AU 652610 B	01-09-94
		AU 1144292 A	10-09-92
		CA 2061636 A	06-09-92
		IL 101115 A	10-01-97
		JP 5095789 A	20-04-93
		US 5510471 A	23-04-96
		US 5633448 A	27-05-97

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	MOLECULAR AND GENERAL GENETICS, vol. 236, 1993, pages 315-325, XP002016640 TEMPLE, S.J., ET AL.: "Modulation of glutamine synthetase gene expression in tobacco by the introduction of an alfalfa glutamine synthetase gene in sense and antisense orientation: molecular and biochemical analysis" see the whole document ---	3
A	EP 0 508 909 A (RHONE POULENC AGROCHIMIE) 14 October 1992 see the whole document -----	1-8

1

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

United States Patent and Trademark Office
(Box PCT)
Crystal Plaza 2
Washington, DC 20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing: 16 October 1997 (16.10.97)	
International application No.: PCT/EP97/01741	Applicant's or agent's file reference: 1996/M206
International filing date: 08 April 1997 (08.04.97)	Priority date: 11 April 1996 (11.04.96)
Applicant: DONN, Günter et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International preliminary Examining Authority on:

25 September 1997 (25.09.97)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

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Authorized officer:

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